

## **REMARKS/ARGUMENTS**

### **Status of the claims**

Applicants thank the Examiner for indicating that claims 45-57, 60-62, 65-69 and 83 are enabled for how to make the instantly claimed compounds. Claims 1-44, 58-59, 63-64 and 70-82 remain canceled. Claims 45-57, 60-62, 65-69 and 83 are currently pending.

### **Claim Rejections under 35 U.S.C. §112, First paragraph**

Claims 45-57, 60-62, 65-69 and 83 were rejected as lacking enablement. The Examiner alleges that the specification does not reasonably provide enablement for the use of the instantly claimed compounds of the formula recited in claim 45 for the claimed methods for reducing anxiety by increasing ion flow through KCNQ potassium channel. Applicants respectfully rebut the rejection.

The instant application does teach how to use the compound subject matter of the claims for reducing anxiety by increasing ion flow through KCNQ potassium channel. Applicants submit along with this Amendment a Rule 1.132 Declaration signed by inventor Dr. Gregory C. Rigdon and its accompanying attachments, Exhibits A, B and C. Exhibit A is a Curriculum Vitae of Gregory C. Rigdon. Exhibit B are KCNQ2/3 activity data for the compounds of formula recited in claim 45. Exhibit B has clearly demonstrated that the compounds of the formula recited in claim 45 exhibit good activity in modulating KCNQ potassium channels by increasing ion flow through the potassium channels. Exhibit C presents a detailed study of anxiolytic activity effects of compound ICA-27243 in the Geller conflict model in rats. For example, compound ICA-27243 produces an increase in punished lever pressing in the rat, with a maximum mean increase of 55% (see paragraph 13 of the Declaration). In the Declaration, Dr. Rigdon states that the compound used in Example 6 of the present application is ICA-27243, whose structure is shown as compound 4 in Figure 7 of the instant application (see paragraph 15 of the Declaration). Therefore, Exhibit C and Example 6 have clearly demonstrated the anxiolytic activity of compounds of the formula recited in claim 45 and the use of the compounds to reduce anxiety.

In the Declaration, Dr. Rigdon further states that the assays for determining whether or not a selected compound acts as a KCNQ channel opener, the assays for measuring ion flux and the assays to test potassium channel openers for their ability of treating anxiety are disclosed in the specification. These assays are well-known in the art and are simple and routine operations (see paragraphs 6-10 of the Declaration). There, it is well within the capabilities of those skilled in the art to routinely test compounds for their ability to open KCNQ potassium channels and to treat anxiety (see paragraph 11 of the Declaration). Consequently, there exists a nexus between the identification of compounds of the formula recited in claim 45 as anxiolytics and their KCNQ channel modulating abilities (see paragraph 16 of the Declaration). A person of ordinary skill in the art is able to determine the ability of the KCNQ potassium channel openers of the formula recited in claim 45 to treat anxiety in a subject without undue experimentation (see paragraphs 17-18 of the Declaration). In view of the object evidence, the use of compounds of the formula recited in claim 45 for the methods for reducing anxiety by increasing ion flow through a KCNQ potassium channel is clearly enabled.

Accordingly, Applicants respectfully request that the enablement rejection be withdrawn.

### CONCLUSION

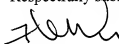
In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

Appl. No. 09/939,230  
Amdt. dated July 10, 2009  
Reply to Office Action of March 11, 2009

PATENT

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,



Zhe Wu  
Reg. No. 52,377

TOWNSEND and TOWNSEND and CREW LLP  
Two Embarcadero Center, Eighth Floor  
San Francisco, California 94111-3834  
Tel: 925-472-5000  
Fax: 415-576-0300  
Attachments  
ZW:  
62072044 v1

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of:

Alan David Wickenden *et al.*

Application No.: 09/939,230

Filed: August 24, 2001

For: METHODS FOR TREATING OR  
PREVENTING PAIN AND ANXIETY

Examiner: Royds, Leslie A.

Art Unit: 1614

DECLARATION OF GREGORY C.  
RIGDON UNDER 37 CFR 1.132

Mail Stop AF  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

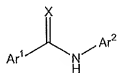
I, Gregory C. Rigdon, state as follows:

1. I am currently employed by Icagen Inc. as a Vice President of New Product Development. I received a Ph.D. in pharmacology in 1985 from the Texas Tech University. I received a B.Sc. in biology in 1980 from the Abilene Christian University in Texas. I have conducted research in the field of pharmaceutical drug discovery for approximately 24 years. I am a co-inventor of the above-referenced patent application. I am also a co-inventor of 2 issued U.S. patents, 3 pending U.S. patent applications and a co-author of 36 scientific publications. A copy of my Curriculum Vitae (CV) is attached as Exhibit A to this declaration.

2. I have read and am familiar with the contents of the above-referenced patent application. In addition, I have read the Office Action, dated March 11, 2009, received from the United States Patent & Trademark Office in the above-reference patent application.

3. It is my understanding that the Examiner has rejected claims 45-57, 60-62, 65-69 and 83 of the present invention as lacking enablement. The Examiner states that the specification does not reasonably provide enablement for the use of compounds of the formula recited in claim 45 for reducing anxiety by increasing ion flow through KCNQ potassium channels.

4. Claim 45 is directed to methods for reducing anxiety in a subject in need thereof by increasing ion flow through KCNQ potassium channels in a cell by administering to the subject a pharmaceutical composition comprising a compound having the formula:



5. This declaration provides objective evidence to demonstrate that compounds of the formula recited in claim 45 modulate KCNQ potassium channels and function to reduce anxiety such that the use of compounds of the formula recited in claim 45 for reducing anxiety by increasing ion flow through KCNQ potassium channels is enabled. All the work described herein was either conducted by me, at my direction, or by my colleagues who work with me as part of the team of scientists working on this project.

6. The specification sets forth a number of simple assays, which can be used routinely to determine whether or not a selected compound acts as a KCNQ potassium channel opener. The assays involve the *in vivo* or *in vitro* contacting of a sample having a KCNQ channel with a test compound followed by measurement of the KCNQ potassium channel activity. See specification, page 23, lines 25-29. The activity of the test compound may then be compared with untreated control samples. See specification, page 23, lines 27-29. These assays can be conducted using high throughput screening methods and large libraries of chemical compounds, which are well known in

the art. Systematic screening of potential KCNQ channel openers can be aided by robotic automation. See specification, page 25, lines 21-27. KCNQ potassium channel opening activity may be determined by measuring changes in ion flux through detection of cell or membrane polarization. See specification, page 24, lines 4-6. Cell or membrane polarization is detected by measuring changes in current using standard techniques such as voltage clamps or patch clamps. See specification, page 24, lines 6-10.

7. The specification further provides additional routine methods useful in identifying KCNQ channel openers, such as measuring current; measuring membrane potential; measuring ion flux (e.g., potassium or rubidium); measuring potassium concentration; measuring second messengers and transcription levels, using potassium-dependent yeast growth assays; measuring pain responses in mice (e.g., with formalin algesia or hotplate assays); measuring ligand binding; and using, e.g., voltage-sensitive dyes, radioactive tracers, and patch-clamp electrophysiology. See specification, page 23, lines 12-18.

8. Routine assays for measuring ion flux, including those involving the measurement of potassium or rubidium ion flux by directly detecting the concentration changes of the ions (e.g., radioisotopic labeling) are also disclosed in the specification. See specification, page 23, lines 23-32. In addition, ion flux may be measured by determining changes in physiological conditions, such as transmitter release (e.g., dopamine), hormone release (e.g., insulin), transcriptional changes to both known and uncharacterized genetic markers (e.g., northern blots), cell volume changes (e.g., in red blood cells), immunoresponses (e.g., T cell activation), changes in cell metabolism such as cell growth or pH changes, and changes in intracellular second messengers such as  $\text{Ca}^{2+}$ , or cyclic nucleotides. See specification, page 24, line 30 to page 25, line 8.

9. The specification also sets forth simple and routine assays to test potassium channel openers for their ability of treating anxiety, such as the standard Geller conflict procedure, which is an art-accepted model for testing anxiety compounds. See specification, page 35, Example 6.

10. The above assays were all well known in the art at the time of filing the application, as evidenced by the references cited therein (Ackerman *et al.*, *New Engl. J. Med.* 336:1575-1595 (1997); Hamil *et al.*, *Pflugers. Archiv.* 391:85 (1981); Vestergaard-Bogind *et al.*, *J. Membrane Biol.* 88:67-75 (1988); Daniel *et al.*, *J. Pharmacol. Meth.* 25:185-193 (1991); Holevinsky *et al.*, *J. Membrane Biology* 137:59-70 (1994); Blatz *et al.*, *Nature* 323:718-720 (1986); and Park, *J. Physiol.* 481:555-570 (1994)).

11. Therefore, it is well within the capabilities of those skilled in the art to routinely test compounds for their ability to open KCNQ potassium channels and to treat anxiety (e.g. using the Geller conflict procedure).

12. Enclosed as Exhibit B are KCNQ2/3 activity data for the compounds of the formula recited in claim 45. The EC50 values are expressed in micromolar. The compounds in Exhibit B correspond to those in Figure 7 of the specification. As demonstrated in Exhibit B, the compounds of the formula recited in claim 45 have good activity in modulating KCNQ potassium channels by increasing ion flow through the potassium channels.

13. Enclosed as Exhibit C is a detailed study of anxiolytic activity effects of compound ICA-27243 in the Geller conflict model in rats. Figure 1 in Exhibit C is also duplicated below. Figure 1 in Exhibit C presents the percent change in response over baseline for both punished and unpunished lever presses for all doses of ICA-27243. As shown and described in Exhibit C, compound ICA-27243 produces an increase in punished lever pressing in the rat, with a maximum mean increase of 55%.<sup>1</sup> Exhibit C has demonstrated the anxiolytic activity of compounds of the formula recited in claim 45 and the use of the compounds to reduce anxiety.

---

<sup>1</sup> The anxiolytic activity of ICA-27243 is also described in the results and discussion sections of Exhibit C.

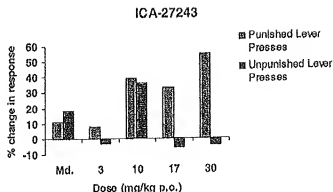


Figure 1 in Exhibit C

14. The dates of the enclosed Exhibit C have been redacted. The redacted dates are prior to August 24, 2001, the filing date of the present application.

15. The Examiner stated that the identity of the compound in Example 6 of the present application was not set forth. I submit that the compound used in Example 6 of the present application is ICA-27243, whose structure is shown as compound 4 in Figure 7 of the instant application.

16. Based on my experiments and scientific opinion, there exists a nexus between the identification of compounds of the formula recited in claim 45 as anxiolytics and their KCNQ channel modulating abilities.

17. It is also my scientific opinion that one skilled in the art, using the teachings in the specification and methods generally known in the art coupled with the scientific data provided in Exhibits B and C and the specification would be able to determine the ability of the KCNQ potassium channel openers of the formula recited in claim 45 to treat anxiety in a subject.

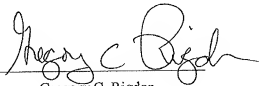
18. In view of the foregoing, I submit that one skilled in the art is able to use compounds of the formula recited in claim 45 for the methods for reducing anxiety

by increasing ion flow through a KCNQ potassium channel without undue experimentation.

19. I further declare that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: \_\_\_\_\_

19 June 2009

  
\_\_\_\_\_  
Gregory C. Rigdon



## UNITED STATES PATENT AND TRADEMARK OFFICE

018512-006610US

UNITED STATES DEPARTMENT OF COMMERCE  
United States Patent and Trademark Office  
Address: COMMISSIONER FOR PATENTS  
P.O. Box 1450  
Alexandria, Virginia 22313-1450  
www.uspto.gov

FJM/WC

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/939,230	08/24/2001	Alan David Wickenden	018512-006610US	5203

20350 7390 03/11/2009  
TOWNSEND AND TOWNSEND AND CREW, LLP  
TWO EMBARCADERO CENTER  
EIGHTH FLOOR  
SAN FRANCISCO, CA 94111-3834

MAR 11 2009 EXAMINER  
ROYDS, LESLIE A

ART UNIT	PAPER NUMBER
1614	

MAIL DATE	DELIVERY MODE
03/11/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Seq. Listing May be in action-If  
further docketing required  
please advise

Response Due 5/11/09  
2 Mos. (optional) 6/11/09  
Initial Due Date 6/11/09  
Final Due Date 9/11/09

SLK

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.  
Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME <b>Greg C. Rigdon</b>		POSITION TITLE <b>Vice President</b>	
eRA COMMONS USER NAME			
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Abilene Christian University, Abilene, TX	BS	1980	Biology
Texas Tech University, Lubbock, TX	PhD	1985	Pharmacology

### A. Positions and Honors:

1985-1986	National Research Council Research Associate, EPA, Research Triangle Park, NC
1986-1987	Fellow, Dept. of Pharmacology, Burroughs Wellcome Co., RTP, NC
1987-1988	CNS Screening Coordinator, Burroughs Wellcome Co., RTP, NC
1988-1994	Head, Neuropharmacology Lab, Burroughs Wellcome Co., RTP, NC
1994-1995	Leader, Preclinical Psychiatry, Burroughs Wellcome Co., RTP, NC
1996-1997	Principal Clinical Research Scientist, CNS Clinical Res., Glaxo Wellcome, Inc., RTP, NC
1997	International Product Development Team Leader, Glaxo Wellcome, Inc., RTP, NC
1997-2000	Director, New Product Development, Icagen Inc., RTP, NC
2000-2001	Senior Director, New Product Development, Icagen Inc., RTP, NC
2001-present	Vice President, New Product Development, Icagen Inc., RTP, NC

### Honors and Awards

1984	National Dean's Honor List
1985	National Research Council Research Associateship Award

### Professional Societies

Society for Neuroscience  
American Society for Pharmacology and Experimental Therapeutics  
General Pharmacology/Safety Pharmacology Society  
Drug Information Association

### B. Selected peer-reviewed publications (in chronological order)

1. Peterson, Steven L., Napier, T. Celeste, Rigdon, Greg C. and Pirch, James H. 1983. Dissimilar responses of cortical neurons to chronic trazodone or desipramine treatment. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 7: 175-181.
2. Pirch, James H., Corbus, Mary Jo, and Rigdon, Greg C. 1983. Single-unit and slow potential responses from rat frontal cortex during associative conditioning. *Experimental Neurology* 82: 118-130.
3. Pirch, James H., Lyness, William H., Corbus, Mary Jo, and Rigdon, Greg C. 1984. Pharmacological and other approaches for investigation of neurochemical substrates of event-related slow potentials. In *Substrates of Event-Related Slow Potentials. Seventh International Conference on Event-Related Potentials of the Brain.*
4. Rigdon, Greg C. and Pirch, James H. 1984. Microinjection of GABA or procaine into the nucleus basalis magnocellularis affects cue-elicited unit responses in the rat frontal cortex. *Experimental Neurology* 85: 283-296.
5. Pirch, James H., Corbus, Mary Jo and Rigdon, Greg C. 1985. Conditioning-related single unit activity in the frontal cortex of urethane anesthetized rats. *International Journal of Neuroscience* 25: 263-271.
6. Pirch, James H., Corbus, Mary Jo, Rigdon, Greg C. and Lyness, William H. 1985. Nucleus basalis cholinergic neurons and cortical event-related potentials in the rat. In *Senile Demential of the Alzheimer Type*. pp. 231-242. ed. Hutton, J. T. and Kenney, A. D. New York. Alan R. Liss, Inc.

7. Pirch, James H., Corbus, Mary Jo, Rigdon, Greg C. and Lyness, William H. 1986. Generation of cortical event-related slow potentials in the rat involves nucleus basalis cholinergic innervation. *Electroencephalography and Clinical Neurophysiology* 63: 464-475.
8. Rigdon, Greg C., and Pirch, James H. 1986. Nucleus basalis involvement in conditioned neuronal responses in the rat frontal cortex. *Journal of Neuroscience* 6: 2535-2542.
9. Dyer, Robert S. and Rigdon, Greg C. 1987. Urethane affects the rat visual system at subanesthetic doses. *Physiology and Behavior* 41: 327-330.
10. Rigdon, Greg C. and Dyer, Robert S. 1987. Ontogeny of flash evoked potentials in awake, unanesthetized rats. *Int. J. Dev. Brain Res.* 5: 447-454.
11. Rigdon, Greg C. and Dyer, Robert S. 1987. Ketamine alters rat flash evoked potentials. *Pharmacol. Biochem. Behav.* 30: 421-426.
12. Rigdon, Greg C., Boyes, William K. and Dyer, Robert S. 1989. Effect of perinatal monosodium glutamate administration on visual evoked potentials of juvenile and adult rats. *Neurotox. Teratol.* 11: 121-128.
13. Pirch, James H., Rigdon, Greg C., Rucker, Herbert K. and Turco, Karen. Basal forebrain modulation of cortical cell activity during conditioning. In: *The Basal Forebrain: Anatomy to Function*. Ed. by: Napier, Kalivas and Hanin. Plenum Press, 1990.
14. Rigdon, Greg C. 1990. Differential effects of apomorphine on prepulse inhibition of acoustic startle in two rat strains. *Psychopharmacology* 102:419-421.
15. Rigdon, Greg C. and Wang, Ching M. 1991. Serotonin uptake blockers inhibit the firing of presumed serotonergic dorsal raphe neurons *in vitro*. *Drug Dev. Research* 22:135-140.
16. Rigdon, Greg C. and Viik, Kaido. 1991. Prepulse inhibition as a screening test for potential antipsychotics. *Drug Dev. Research* 23:91-99.
17. White, H.L., Beek, O., Rigdon, G.C., Kraemer, G.W. and Cooper, B.R. 1991. Biochemical and pharmacological properties of BW 1370U87-a novel, selective monoamine oxidase-A inhibitor. *European College of Neuropsychopharmacology*.
18. Burchall, Christopher J., Soroko, Francis E. and Rigdon, Greg C. 1992. Potentiation of the behavioral effects of 5-hydroxytryptophan by BW 1370U87, a selective monoamine oxidase-A inhibitor. *Drug Development Research* 25:209-213.
19. Cooper, B.R., White, H.L., Beek, O., Norton, R.M., Rigdon, G.C., Howard, J.L., Kraemer, G.W., and Ferris, R.M. 1992. Overview of the CNS pharmacology of BW 1370U87: A chemically novel, reversible, selective MAO-A inhibitor with potential to be a new antidepressant drug. *Drug Development Research* 25:181-190.
20. Rigdon, Greg C. and Weatherspoon, John K. 1992. 5-HT<sub>1A</sub> receptor agonists block prepulse inhibition of acoustic startle reflex. *Journal of Pharmacology and Therapeutics* 263:486-493.
21. Chang, Kwen-Jen, Rigdon, Greg C., Howard, James L. and McNutt, Robert W. 1993. A novel, potent and selective nonpeptidic delta-opioid receptor agonist BW 373U86. *Journal of Pharmacology and Experimental Therapeutics*. 267:852-857.
22. Norman, Mark H., Rigdon, Greg C., Navas, Frank and Cooper, Barrett R. 1994. Cyclic benzamides as mixed dopamine D<sub>2</sub>/serotonin 5-HT<sub>2</sub> receptor antagonists: new atypical antipsychotic agents. *J. Med. Chem.* 37:2552-63.
23. Ferris, R.M., Brieady, L., Mehta, N., Hollingsworth, E., Rigdon, G.C., Wang, C., Soroko, F., Wastila, W. and Cooper, B.R. 1995. Pharmacological properties of 403U76 a new chemical class of selective serotonin reuptake inhibitor. *J. Pharm. Pharmacol.* 47:775-781.
24. Brieady, L., Mehta, N.B., Koble, C., Hollingsworth, E., Rigdon, G.C., Soroko, F. and Ferris, R.M. 1994. Diphenyl sulfides as selective and specific inhibitors of serotonin reuptake. *J. Med. Chem.*
25. Durcan, M., Rigdon, G.C., Morgan, P. and Norman, M.H. 1995. Is clozapine selective for the dopamine D<sub>4</sub> receptor? *Pharmacology Letters*. 57:PL275-283.
26. Norman, M.H., Rigdon, G.C., Navas, F. and Hall, W. 1995. Structure-activity relationships within a series of substituted benzamides: potent D<sub>2</sub>/5-HT<sub>2</sub> antagonists as neuroleptic Agents. *J. Med. Chem.*
27. Norman, M.H., Minick, D.J. and Rigdon, G.C. 1996. Effect of linking bridge modifications on the antipsychotic profile of some phthalimide and isoindolinone derivatives. *J. Med. Chem.* 39:149-157.
28. Rigdon, G.C., Norman, M. H., Cooper, B.R., Howard, J.H., Boncek, V.M., Faison, W.L., Nanny, K.P. and Pollard, G.T. 1996. 1192U90 in animal tests that predict antipsychotic efficacy, anxiolysis and extrapyramidal side effects. *Neuropsychopharmacology* 15: 231-242.

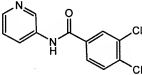
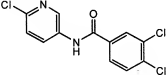
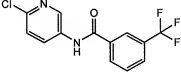
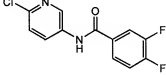
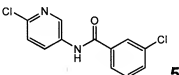
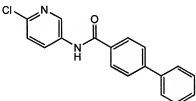
29. White, H.L., Scates, P.W., Harrelson, J.C., Johnson, T.E., Norton, R.M., Jones, S.A., Rigdon, G.C., Hughes, J.E., Cooper, B.R., and Harfenist, M. 1998. Biochemical and pharmacologic properties of 2614W94, a reversible, competitive inhibitor of monamine oxidase-A. *Drug Dev. Res.* 45:1-9.
30. Musso DL, Cochran FR, Kelley JL, McLean EW, Selph JL, Rigdon GC, Orr GF, Davis RG, Cooper BR, Styles VL, Thompson JB, Hall WR. Indanylidenes. 1. Design and synthesis of (E)-2-(4,6-difluoro-1-indanylidene)acetamide, a potent, centrally acting muscle relaxant with antiinflammatory and analgesic activity. *J Med Chem.* 2003 Jan 30;46(3):399-408
31. Musso, D.L., Orr, G.F., Cochran, F.R., Kelley, J.L., Selph, J.L., Rigdon, G.C., Cooper, B.R., and Jones, M.L. Indanylidenes Part II. The Design and Synthesis of (E)-2-(4-chloro-6-fluoro-1-indanylidene)-N-methylacetamide, A Potent Anti-inflammatory and Analgesic Agent Without Centrally Acting Muscle Relaxant Activity. *J Med Chem.* 2003 Jan 30;46(3):409-16.
32. Wickenden, A.D., Roeloffs, R., McNaughton-Smith, G., and Rigdon, G.C. KCNQ2/Q3 Potassium Channels: Drug Targets For The Treatment Of Epilepsy And Pain. *Expert Opinion on Therapeutic Patents.* 2004 14(4)
33. McNaughton-Smith G.A., Burns J.F., Stocker J.W., Rigdon G.C., Creech C., Arrington S., Shelton T., de Franceschi L. Novel inhibitors of the Gardos channel for the treatment of sickle cell disease. *J Med Chem.* 2008 Feb 28;51(4):976-82.
34. Roeloffs R., Wickenden A.D., Crean C., Werness S., McNaughton-Smith G., Stables J., McNamara J.O., Ghodadra N., Rigdon G.C. In vivo profile of ICA-27243 [N-(6-chloro-pyridin-3-yl)-3,4-difluoro-benzamide], a potent and selective KCNQ2/Q3 (Kv7.2/Kv7.3) activator in rodent anticonvulsant models. *J Pharmacol Exp Ther.* 2008 Sep;326(3):818-28.
35. Ataga KI, Smith WR, De Castro LM, Swerdlow P, Saunthararajah Y, Castro O, Vichinsky E, Kutlar A, Orringer EP, Rigdon GC, Stocker JW; ICA-17043-05 Investigators. Efficacy and safety of the Gardos channel blocker, senicapoc (ICA-17043), in patients with sickle cell anemia. *Blood.* 2008 Apr 15;111(8):3991-7.
36. Wickenden AD, Krajewski JL, London B, Wagoner PK, Wilson WA, Clark S, Roeloffs R, McNaughton-Smith G, Rigdon GC. N-(6-chloro-pyridin-3-yl)-3,4-difluoro-benzamide (ICA-27243): a novel, selective KCNQ2/Q3 potassium channel activator. *Mol Pharmacol.* 2008 Mar;73(3):977-86.

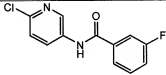
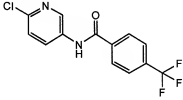
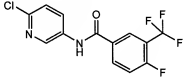
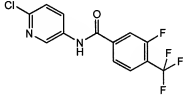
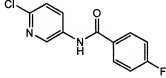
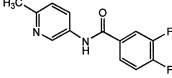
#### Patents:

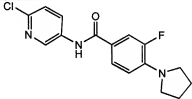
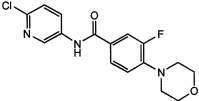
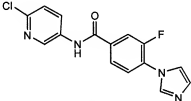
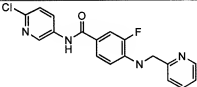
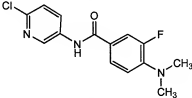
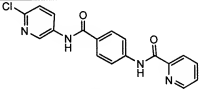
1. Kelley, J.L., Rigdon, G.C., Cooper, B.R., McLean, E.W., Musso, D.L., Orr, G.F. Selph, J.L. and Styles, V.L. Amide Derivatives and Their Therapeutic Use. Filed 11 May, 1993, United Kingdom.
2. McNaughton-Smith, G.A., Rigdon, G.C., and Stocker, J.W. Gardos Channel Antagonists. Filed February 23, 1999, United States.
3. Wickended, A.D., Rigdon, G.C. and McNaughton-Smith, G. Methods for treating and preventing pain and anxiety. Filed August 4, 2000.
4. Rigdon, G.C., Stocker, J.W., and McNaughton-Smith, G. Methods for Treating Diseases Related to Intraocular Pressure. Filed on April 16, 2002.
5. Wickended, A.D., Rigdon, G.C., McNaughton-Smith, G., Grant A., Gross, M. F. Methods for treating or preventing pain and anxiety. Filed August 24, 2001.
6. Castle, N. A., Rigdon, G.C., Krafte, D. S. Treatment Methods Using Triaryl Methane Compounds. Filed September 19, 2008.

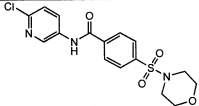
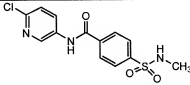
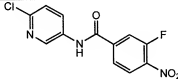
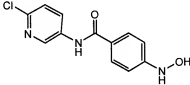
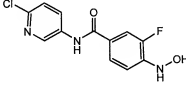
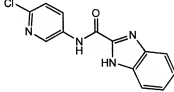
#### **C. Research Support**

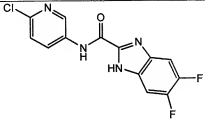
None listed

Compound	KCNQ2/3_EC50 (μM)
 <p><b>1</b></p>	6.3089
 <p><b>2</b></p>	0.6672
 <p><b>3</b></p>	0.3983
 <p><b>4</b></p>	0.3807
 <p><b>5</b></p>	0.266
 <p><b>6</b></p>	0.2646

Compound	KCNQ2/3_EC50 (μM)
 <p style="text-align: center;"><b>9</b></p>	1.0616
 <p style="text-align: center;"><b>10</b></p>	0.3194
 <p style="text-align: center;"><b>11</b></p>	0.4155
 <p style="text-align: center;"><b>12</b></p>	0.326
 <p style="text-align: center;"><b>13</b></p>	0.9821
 <p style="text-align: center;"><b>8</b></p>	6.3117

Compound	KCNQ2/3_EC50 (μM)
 <p><b>30</b></p>	0.684
 <p><b>31</b></p>	2.2808
 <p><b>32</b></p>	1.241
 <p><b>33</b></p>	0.699
 <p><b>34</b></p>	1.758
 <p><b>35</b></p>	0.1575

Compound	KCNQ2/3_EC50 (μM)
 <p><b>36</b></p>	1.412
 <p><b>40</b></p>	5.062
 <p><b>37</b></p>	0.51
 <p><b>38</b></p>	1.701
 <p><b>39</b></p>	3.157
 <p><b>45</b></p>	3.998

Compound	KCNQ2/3_EC50 (μM)
 <p style="text-align: center;"><b>48</b></p>	7.026



## Effects of ICA-27243 in the Geller Conflict Model in the Rat – a Model of Anxiolytic Activity

**Study Identification:** IR-A22

**Test Article:** ICA-27243

**Performing Laboratory:** Howard Associates  
MCB/HLB Complex, Research Triangle Institute  
P.O. Box 12194  
Research Triangle Park, NC 27709

**Sponsor Address:** ICAgen, Inc.  
P.O. Box 14487  
Research Triangle Park, NC 27709

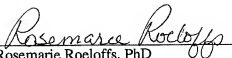
**Author(s):** Rosemarie Roeloffs

**Purpose:** To determine whether ICA-27243 produces anxiolytic activity in the Geller conflict model in the rat.

Final

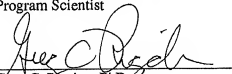
ICAgen, Inc.  
Ion Channel Advances  
P.O. Box 14487  
Research Triangle Park, NC 27709

Effects of ICA-27243 in the Geller Conflict Model in the Rat – a Model of Anxiolytic Activity



Rosemarie Roeloffs, PhD  
Program Scientist

Date



Greg C. Rigdon, PhD  
Sr. Director, New Product  
Development

Date

## Table of Contents

### Summary

#### I. Introduction

#### II. Methods

- 2.1 Subjects
- 2.2 Test Article
- 2.3 Compound Preparation and Administration
- 2.4 Equipment
- 2.5 Procedure
- 2.6 Data Analysis

#### III. Results

#### IV. Discussion and Conclusions

Figure 1. Effects of ICA-27243 and Chlordiazepoxide on Punished and Unpunished Lever Pressing in the Rat

Appendix 1 Howard Associates Study Report – October 9, 2000

## Effects of ICA-27243 in the Geller Conflict Model in Rats – a Model of Anxiolytic Activity

### Summary

The objective was to determine whether ICA-27243 possesses anxiolytic activity in the Geller conflict model in the rat.

Doses of 3, 10, 17, 30, and 56 mg/kg of ICA-27243 were administered p.o.

ICA-27243 at 17, 30, and 56 mg/kg produced an anxiolytic-like effect, with a maximum increase of 55% over baseline.

## 1. Introduction

The objective of this study was to determine whether ICA-27243 produces an anxiolytic-like effect when tested in the Geller conflict model in rats. Chlordiazepoxide, a benzodiazepine, was also tested as an active control for this experiment.

## 2. Methods

### 2.1 Subjects

Six male CD rats from Charles River Laboratories, Raleigh, NC, were maintained at one or two per cage on a reverse light/dark cycle (light on 1800-0600) in the Research Triangle Institute Animal Facility with ad libitum water. Food was restricted to 13 grams per day to maintain motivation to press a lever for food. Testing was conducted during the period of June 9 to October 3, 2000, by Howard Associates, LLC, Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC. (Study report found in Appendix 1)

### 2.2 Test Article

The test article, ICA-27243.3 was synthesized at ICAGEN, Inc., Durham, North Carolina.

Date Registered: March 21, 2000  
Batch: 6  
Notebook: GMS/58/91  
Purity: >95%

### 2.3 Compound Preparation and Administration

ICA-27243 was homogenized in 0.5% methyl cellulose and administered p.o. in a volume of 1 mL/kg 15 minutes before testing. Chlordiazepoxide HCL (Research Biochemicals International, Natick, MA), the positive control, was dissolved in 0.9% saline and administered similarly. 0.5% methyl cellulose was the vehicle control. Doses of ICA-27243 were tested in the order 3, 10, 30, 56, and 17mg/kg during the period of June 9 through June 23, 2000. Doses of chlordiazepoxide were tested in the order 17, 10, 3, 5.6, and 30mg/kg, and then vehicle, during the period of September 8 to October 3, 2000. Baseline controls were tested on Mondays and Thursdays, ICA-27243 and chlordiazepoxide doses were tested on Tuesdays and Fridays.

### 2.4 Equipment

Each of six operant chambers inside sound-attenuating enclosures (Coulbourn Instruments, Allentown, PA) was equipped with a pellet dispenser (45-mg food pellets, BioServ, Frenchtown, NJ), an intelligence panel with a house light at the upper center, a feeder bin at the lower center, a lever manipulandum at the lower left, and a cue light (three bulbs functioning together) just above the lever, and a grid floor to which a Coulbourn shocker delivers 60-HZ

500-msec pulses through a custom-made current intensity stepper. Control and data acquisition are done by a Data General NOVA 3/12 minicomputer via an InterAct interface.

## 2.5 Procedure

Baseline: The subjects had been trained to press a lever for food on a multiple schedule consisting of four periods of variable-interval reinforcement, in which a pellet was delivered for a lever press every 2 minutes on the average (randomly assigned intervals no longer than 4 minutes), and four interspersed 3-minute periods of fixed-ratio (FR) 1 reinforcement, with the cue light on, in which a pellet was delivered for each lever press. Each lever press in FR1 also delivered an electric shock to the grid floor (the 'conflict' or 'punishment' period) with the following limitations: The first lever-press in conflict produced no shock, the second lever-press produces 0.1 mA, and the intensity was increased by 0.1 mA for each lever-press thereafter; the intensity was reset to 0.00 mA at the beginning of each of the four conflict periods of a session. The subjects had been given chlordiazepoxide at behaviorally active doses on eight occasions before the beginning of compound and positive control testing, to make sure that they showed a reliable increase in punished lever pressing under the influence of a standard benzodiazepine anxiolytic.

## 2.6 Data Analysis

Punished and unpunished lever pressing data were analyzed separately, as were data for the two treatments (ICA-27243 and chlordiazepoxide). For each subject, the median of the values on the days before each treatment day was taken as baseline control for that compound: 5 days for ICA-27243, 6 days for chlordiazepoxide and vehicle; that is, the baseline control was for the same calendar period as the treatment. Values for the five doses of ICA-27243 were submitted to within-subject analysis of variance, and values for the five doses of chlordiazepoxide and vehicle were analyzed similarly. Where a main effect was found, each dose was compared to baseline by the Newman-Keuls test. An effect was considered significant if  $p < 0.05$ .

## 3. Results

Table 1 in the Appendix (Howard Associates Study Report – October 9, 2000) gives baseline medians and values for all test days for both punished and unpunished lever presses.

Figure 1 presents the percent change in response over baseline for both punished and unpunished lever presses for all doses of ICA-27243 and chlordiazepoxide.

ICA-27243 changed punished lever pressing:  $F(5, 30) = 8.65$ . Seventeen, 03, and 56 mg/kg were greater than baseline, with a maximum mean increase of 55%. The compound also changed unpunished lever pressing:  $F(5, 30) = 3.64$ . However, the changes were irregular, and no dose differed significantly from baseline. At 30 mg/kg, all five subjects with relatively high baselines showed decreases in unpunished lever pressing; at 56 mg/kg, one subject showed a large decrease and three of the other four with high baselines showed decreases; thus the highest doses were near the threshold for a non-specific reduction in behavior.

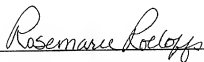
The positive control, chlordiazepoxide, changed punished lever pressing:  $F(6, 36) = 21.99$ . The highest four doses were greater than baseline, with a maximum mean increase of 120%. Chlordiazepoxide also changed unpunished lever pressing:  $F(6, 36) = 3.00$ . Here, too, no dose differed from baseline, although the values for 3 to 17 mg/kg were substantially above baseline. Values for some subjects at 30 mg/kg suggest a non-specific reduction in behavior. Vehicle (0.5% methyl cellulose) showed no tendency to affect punished or unpunished lever pressing.

#### 4. Discussion and Conclusion

In the Geller Conflict model, an increase in punished lever pressing is an indication of anxiolytic activity. Sedative-hypnotic anxiolytics of the benzodiazepine and barbiturate classes, as well as ethanol, register positive in this model. Anxiolytics that require many days or weeks of treatment to produce efficacy, such as buspirone and tricyclic antidepressants, do not test positive in this model.

The positive control in this study, chlordiazepoxide, a benzodiazepine, produced a large increase in punished lever pressing (maximum of 120% over baseline). ICA-27243 also produced an increase in punished lever pressing (maximum of 55% over baseline), although not as large an increase as observed with chlordiazepoxide.

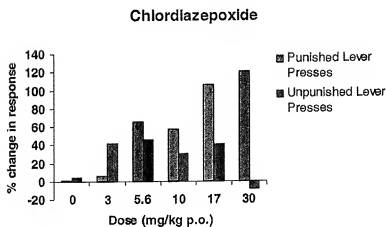
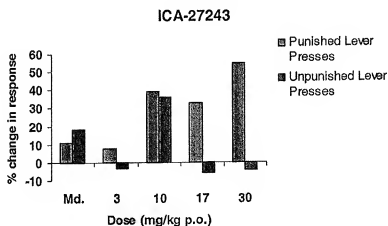
These results suggest that ICA-27243 could have immediate-onset anxiolytic activity.



Rosemarie Roeloffs, Ph.D.  
Study Director

Date

Figure 1. Effects of ICA-27243 and Chlordiazepoxide on Punished and Unpunished Lever Pressing in the Rat



**Final**

## **Appendix 1 Howard Associates Study Report – October 9, 2000**